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Diversity of Filamentous Fungi of Area from Brazilian Caatinga and High-Level Tannase Production Using Mango (*Mangifera indica* L.) and Surinam Cherry (*Eugenia uniflora* L.) Leaves under SSF

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ABSTRACT

Tannase is a biotechnologically important enzyme that can be produced during fungal fermentation of organic matter. The Caatinga is an exclusive Brazilian ecosystem that has been largely unexplored by science, particularly its filamentous fungal diversity. This study evaluated the diversity of filamentous fungi in the Caatinga soils of Pernambuco, Brazil, and their potential for tannase production by solid-state fermentation (SSF) of mango (*Mangifera indica* L.) and Surinam cherry (*Eugenia uniflora* L.) leaves. A total of 4711 isolates were obtained, 2090 during the rainy seasonand 2621 during the dry season. The isolates belonged to 18 genera and 66 species, with *Aspergillus* and *Penicillium* having the highest species richness. The dry season had a higher diversity index. *Aspergillus* was the dominant genus, and *A. flavus*, *A. sclerotiorum*, and *A. ochraceus* the most abundant species. A representative of each species was tested for tannase production using dried mango and Surinam cherry leaves as substrates; the leaves contained 14.28 and 7.0 g/L tannin, respectively. Most fungal species produced tannase, but the highest yields were obtained when mango leaves were used as substrate for *Penicillium restrictum* (accession URM 6044), *Aspergillus flavofurcatus* (URM 6142), and *A. stromatoides* (URM 6609), which produced 104.16, 87.51, and 81.83 U/mL tannase, respectively. These yields exceeded previously published reports. Filamentous fungi from Caatinga soils have great potential for producing tannase by SSF, and low-cost mango leaves make excellent substrate.

Keywords: Caatinga; Filamentous Fungi; Tannase; Solid-State Fermentation; *Mangifera indica* L.

1. Introduction

The Caatinga is an ecosystem unique to Brazil that is home to many endemic species of plants, animals, and microorganisms. It has a semi-arid climate, with high temperatures and low rainfall, between 200 and 800 mm per year [1]. The soils, with rare exceptions, are very shallow, not flooded, mineralogically rich, stony, and with poor water-holding capacity [2]. Most rainfall in the Caatinga (50% - 70%) is concentrated in three consecutive months, with long periods of drought common [1]. The National Park Catimbau (Catimbau Valley) is an important region of the Caatinga of Pernambuco, Brazil, considered the second largest archaeological park in Brazil. This fully-protected conservation area was created in 2002 [3]. The park is an excellent resource for both archaeological and ecological studies, especially for studies of filamentous fungi of biotechnological interest, where unusual or new to science species are found.

Filamentous fungi comprise a diverse group of microscopic forms that are widely distributed in nature [4]. In fact, the vast majority of fungal species probably spend some part of their life cycles as soil filaments and play key roles in cycling organic matter in soil ecosystems [5].

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In addition to their essential role in decomposition, some species of fungi have broad biotechnological potential and are widely used to produce economically-important enzymes [6]. Tannase is one such enzyme [7].

Tannase (tannin acyl hydrolase EC3.1.1.20) hydrolyzes ester linkages and side-hydrolyzable tannins, such as tannic acid, gallic acid, and glucose. Its extracellular production by bacteria, yeasts and filamentous fungi can be induced in the presence of tannic acid. The genera *Aspergillus* and *Penicillium* are excellent producers of this enzyme [8,9]. Tannase has wide utility in the beverage (especially in juice and beer production), cosmetics, pharmaceutical, and chemical industries [10].

Due to the importance of microbial tannase in various industries, production processes are necessary, which will minimize costs, increase yield, and contribute directly to environmental balance. For example, many agro-industrial wastes are commonly discarded into the environment untreated [11]. In this context, solid-state fermentation (SSF) by fungi presents an excellent alternative for producing tannase while simultaneously reducing waste [7]. Because filamentous fungi grow in nature in solid substrates, such as wood, roots, stems, and leaves of plants, in the absence of free water [12].

Successful production of tannase by SSF has been reported using palm kernel cake, bran tamarind seeds, and jambul leaves (*Syzygium cumini*) as carbon sources [12, 13]. This study is the first to assess the diversity of filamentous fungi isolated from Caatinga soils of Northeastern of Brazil and their biotechnological potential for SSF production of tannase using mango leaves (*Mangifera indica* L.) and Surinam cherry (*Eugenia uniflora* L.) as low-cost substrates.

2. Materials and Methods

2.1. Study Area and Sample Collection

Six soil samples were collected at the Catimbau National Park, Buíque, Pernambuco, Brazil (S08˚04'25", W37˚15'52"). Three sets of samples were collected in the dry months of June, July, and August, 2009, when rainfall was 0.0, 11.0, and 0.0 mm, respectively, while three were taken during the rainy season in February, March, and April, 2010, when rainfall was 109.0, 120.0, and 236.0 mm, respectively. At each time, ten soil samples were collected in each of three 4 × 25 m transects at a depth of 0 - 20 cm. The ten samples from each transect were combined to give a single composite sample. A total of 18 samples were obtained (six months × three transects each). Soil temperature was measured using a digital thermometer (UT325 Contemp-São Paulo, Brazil). All samples were transported at room temperature in sterilized thin polyethylene plastic bags to the research laboratory of the URM (University of Recife Mycology) culture collection [14].

2.2. Isolation and Purification

Fungi were isolated using suspension methods [15]. All 18 composite soil samples were suspended in sterile distilled water and successively diluted to a final concentration 1:10,000 g/mL. Each dilution was inoculated into five Petri dishes containing Sabouraud agar medium supplemented with 50 mg/L chloramphenicol (SA-C) and five Petri dishes containing dichloran agar medium with rose bengal supplemented with 50 mg/L chloramphenicol. Overall, 180 Petri dishes of Caatinga soil extracts were obtained. The plates were incubated at 28˚C (±2˚C) for 72 h [14].

To purify fungal isolates, fragments of fungal colonies were transferred to Petri dishes containing SA-C medium. After confirmation of purity, the fungal cultures were maintained on malt extract agar medium (MEA) or on potato dextrose agar medium at 25˚C (±2˚C) for later identification based on specific literature [16-25].

2.3. Soil Analysis

The pH was measured by mixing the soil with water (1:2.5 ratio). The available concentrations of aluminum (Al), calcium (Ca), and magnesium (Mg) were extracted from the soil using 1 M KCl solution in the ratio 1:10 and quantified by titration. Potassium (K), sodium (Na), and phosphorus (P) were extracted with Mehlich solution in a 1:1 (soil:solution) ratio. The K and Na levels were determined by flame photometry (Analyser 910, São Paulo, Brazil), and the P level using a spectrophotometer (Spectrum, SP-1105, São Paulo, Brazil) at 725 nm. The potential acidity (H+Al) was extracted with calcium acetate and quantified by titration [26].

2.4. Comparison of Filamentous Fungal Diversity between the Dry and Rainy Seasons

The diversity of filamentous fungi in the rainy and dry seasons was compared using the Shannon-Wiener index [27] calculated with the program NTSYSpc v. 2.21 [28].

2.5. Principle Component Analysis (PCA)

The original matrix of biotic and abiotic Caatinga was reduced to species that occurred at less than 70% frequency. Then the data were standardized so that parameters with different units could be compared. The Pearson product-moment correlation coefficient between parameters was calculated and used to order the factors to extract eigenvectors and eigenvalues. We projected the first two factors in two-dimensional space, using factors measured outside the sampling sites. The principle compo-

nent analysis (PCA) was conducted in NTSYSpc. 2.21 [28].

2.6. Solid Substrates

Leaves of mango (*Mangifera indica* L.) and Surinam cherry (*Eugenia uniflora* L.) were collected in the Atlantic Forest region in the city of Jaboatão of Guararapes, Pernambuco. The leaves were rinsed with distilled water and dried in an oven at 55˚C for 72 h. The dry material was finely ground in a food processor (Maggiore Pro 1458001, Mallory-Ceará, Brazil) to produce particles of approximately 50 μm, then stored in a dark container at room temperature until the time of fermentation [29].

2.7. Microorganisms

A representative of each isolated species was used in the fermentations. The strains were grown and maintained on MEA slants at 30˚C. Cultures were preserved at 4˚C for short-term storage.

2.8. Inoculum Preparation

Each species isolated, grown for 7 days on MEA was prepared a spore suspension in 10 ml of sterile distilled water with 1% Tween 80. The spores were transferred with the aid of platinum loop. After homogenization, the spores present in the suspension were counted with the aid of a Neubauer chamber [13].

2.9. Moistening Medium and Preparation of SSF Medium for Inoculation

Five grams of each substrate were added separately in Erlenmeyer flasks type. They were then autoclaved at 121˚C for 20 minutes. The medium used to moisten the SSF was prepared salt solution with 0.5% w/v NH_4NO_3, 0.1% w/v and $MgSO_4 \cdot 7H_2O$ 0.1% w/v NaCl and autoclaved at 121˚C for 20 minutes. In each Erlenmeyer flask was added 5 ml of salt solution and 1 ml of the spore suspension. The contents were mixed and then incubated at 30˚C for 96 hours [13].

2.10. Tannin Estimation

Tannin content was estimated following the protein precipitation method [30]. Dried leaves were ground into 50 μm particles in methanol and kept overnight at 4˚C. One mL of extract was mixed with 3 mL of BSA solution and kept for 15 min at room temperature. The tubes were centrifuged at 5000 *g* for 10 min, the supernatant was discarded, and the pellet was dissolved in 3 mL of SDS-triethanolamine solution. One milliliter of $FeCl_3$ solution was added and tubes were kept for 15 min at room temperature for color stabilization. Color was read at 530 nm against a blank.

2.11. Enzyme Extraction

After fermentation, in each Erlenmeyer flask were inserted 50 mL of distilled water containing 0.01% Tween 80, previously sterilized. Then the flasks were shaken on shaker (Tecnal TE421, São Paulo, Brazil) at 150 rpm for 10 min to complete mixing of the contents. Then the crude extract was obtained by filtration using Whatman filter paper number 1. The filtrate was collected in flasks and preserved at 4˚C for later analysis [13].

2.12. Enzyme Assay

Tannase activity was estimated through the formation of a chromogen between gallic acid (released by the action of tannase on methyl gallate) and rhodanine (2-thio-4-ketothiazolidine), in which pink color developed was read at 520 nm using a spectrophotometer (Hitachi-U5100). One unit of tannase activity was defined to the amount of enzyme required to liberate one micromole of gallic acid perminute under defined reaction conditions. Enzyme yield the expressed was units/mL/min [31].

3. Results

We obtained 4711 fungal isolates, 2090 in the rainy season and 2621 in the dry season. The isolates represented 18 genera, including 13 Ascomycota and five Zygomycota, and a total of 66 species. The ascomycotes were: *Acremonium* (1 specie), *Aspergillus* (23), *Chaetomium* (1), *Curvularia* (1), *Eupenicillium* (1), *Fusarium* (3), *Gliomastix* (1), *Neocomospora* (1), *Neosarthorya* (1), *Papulaspora* (1), *Penicillium* (26), *Scopulariopsis* (1), and *Talaromyces* (1). The zygomycotes were: *Absidia* (1), *Gongronella* (1), *Mortierella* (1), *Rhizopus* (1), and *Syncephalastrum* (1). A representative of each species was incorporated into the Catalogue of Micoteca URM Culture Collection (WDCM604) of the Federal University of Pernambuco, Recife, Brazil (**Table 1**).

During the rainy season, the average soil temperature was 26˚C and the dry period, 40˚C. According to the Shannon-Wiener index, the overall diversity of filamentous fungi in the Caatinga was high. However, the dry period had higher diversity (3078 bits·ind^{-1}) than the rainy season (2486 bits·ind^{-1}) (**Figure 1**). The rainy season had greater species richness (53 taxa) than the dry (33) (**Table 1**).

The genera with the highest species richness were *Penicillium* and *Aspergillus*. Although more species (26) of *Penicillium* were present, their populations had few individuals, 99 in the rainy season and 281 in the dry season. *Aspergillus*, with 23 species, had larger populations and was the dominant genus, with 1727 individuals

Table 1. Number of isolates of filamentous fungi species collected during the rainy and dry season in the Caatinga area and activity of Tannase species.

Species	URM number	Caaatinga									Tannaseactivity U/mL (mango leaves)	Tannaseactivity U/mL (surinan cherry leaves)
		Rainy season				Dry season						
		C1	C2	C3	T	C1	C2	C3	T	Total		
Absidia cylindropora Hagem	6031	28	17	13	58	0	0	0	0	58	5.50	3.71
Acremonium terricola (J.H. Mill., Giddens & A.A. Foster) W. Gams	6320	21	17	07	45	30	25	17	72	117	6.60	3.90
Aspergillus aculeatus Lizuga	6603	0	0	0	0	16	16	12	44	44	70.62	4.40
A. awamori Nakaz.	6615	35	29	32	96	0	0	0	0	96	8.94	3.90
A. candidus Link	6607	0	0	0	0	39	34	25	98	98	12.64	4.81
A. avenaceus G. Sm.		0	0	0	0	12	12	12	36	36	3.90	4.48
A. carbonarius (Bainier) Thom	6613	0	0	0	0	19	37	18	74	74	5.99	5.50
A. carneus Smith	6628	0	0	0	0	66	44	33	143	143	53.62	4.43
A. flavofurcatus Bat. & H. Maia	6142	0	0	0	0	76	30	2	108	108	87.51	4.78
A. flavus Link	6602	116	185	232	533	225	252	255	732	1265	68.45	5.25
A. fumigatus Fresen.	6535	175	15	25	215	0	0	0	0	215	18.86	4.48
A. niger Tiegh.	6536	44	36	11	91	65	36	34	135	226	5.52	3.90
A. ochraceus G. Wilh.	6537	130	63	43	236	25	25	27	77	313	49.71	4.48
A. niveus Blochwitz	6604	15	15	15	45	0	0	0	0	45	5.99	3.90
A. parasiticus Speare	5963	90	42	25	157	45	40	36	121	278	56.76	3.90
A. phoenicis (Corda) Thom & Currie	6618	20	20	20	60	0	0	0	0	60	0.78	6.06
A. sclerotiorum G.A. Huber	6635	98	98	98	294	36	44	65	145	361	42.06	0.0
A. stromatoides Raper & Fennel	6609	0	0	0	0	44	47	0	91	91	81.83	0.0
A. sulphureus (Fres.) Thom & Church	6611	0	0	0	0	26	26	0	52	52	12.57	0.0
A. tamarii Kita	5591	0	0	0	0	42	0	0	42	42	27.55	3.78
A. terreus Thom	6614	0	0	0	0	12	14	21	47	47	33.28	4.45
A. terreus var. aureus Thom & Raper	6089	0	0	0	0	34	27	0	61	61	1.38	0.0
A. ustus (Bainier) Thom & Church	6616	0	0	0	0	12	13	8	33	33	24.75	5.25
A. versicolor (Vuill.) Tirab.	6647	0	0	0	0	3	3	2	8	8	8.94	3.80
A. viridinutans Ducker & Thrower	6629	0	0	0	0	9	16	10	35	35	42.36	6.25
Chaetomium cupreum L. M. Ames	6182	0	0	0	0	36	8	0	44	44	0.0	0.0
Curvularia pallescens Boedijn	6601	0	0	0	0	40	13	0	53	53	5.80	3.71
Eupenicillium shaerii Stolk & D.B. Scott	6232	5	5	0	10	0	0	0	0	10	8.58	0.0
Fusarium redolens Wollenw.	6231	0	33	37	70	36	0	0	36	106	3.31	0.0
F. solani (Mart.) Sacc.	5955	9	0	0	9	0	0	0	0	9	5.85	3.90
F. oxysporum E.F. Sm. & Swingle	6228	10	0	0	10	0	0	0	0	10	4.33	3.86
Gliomastix murorum (Corda) S. Hughes	5997	0	0	0	0	0	0	1	1	1	0.00	0.0
Gongronella butleri (Lendn.) Peyronel & Dal Vesco	6174	15	10	0	25	0	0	0	0	25	0.00	0.0
*Mortierella ramanniana*var. *angulispora* (Naumov) Linnem	6145	0	0	0	0	2	0	0	2	2	0.00	0.0
Neocomospora vasinfecta E.F. Sm.	6164	0	0	0	0	3	1	0	4	4	0.00	0.0

Continued

Neosartorya fischeri (Wehmer) Malloch & Cain	6600	0	0	0	0	0	0	3	3	3	0.00	0.0
Papulaspora imersa Host	6622	0	0	1	1	6	6	2	14	15	5.89	3.67
Penicillium adametzii K.M. Zalessky	6275	0	0	0	0	0	0	4	4	15	15.76	5.84
P. aurantiogriseum Dierckx	6026	4	6	6	16	1	0	0	1	4	60.33	4.48
P. canescens Sopp	6044	0	0	0	0	0	3	0	3	17	5. 78	5.22
P. citreonigrum Dierckx	6020	1	0	0	1	4	3	7	14	3	12.64	4.75
P. citrinum Sopp	6224	0	0	0	0	5	7	10	22	15	58.37	6.81
P. decumbens Thom	6620	0	0	0	0	4	3	5	12	22	91.34	5.56
P. funiculosum Thom	6289	0	0	0	0	4	4	4	12	12	5.65	3.70
P. glabrum (Wehmer) Westling	6052	6	4	5	15	8	5	4	17	32	45.14	6.06
P. implicatum Biourge	6223	2	4	2	8	6	7	10	27	31	49.24	6.31
P. janczewskii Zaleski	6041	5	0	0	5	7	8	12	23	32	48.49	5.46
P. lanosum Westling	6288	0	0	0	0	3	3	3	9	9	22.27	4.80
P. lapidosum Raper & Fennell	6042	1	1	0	2	0	0	0	0	2	69.26	5.99
P. lividum Westling	6157	4	4	4	12	7	3	9	19	31	49.71	5.54
P. melinii Thom	6215	0	2	1	3	3	5	1	9	12	51.08	5.46
P. minioluteum Dierckx	6037	1	2	1	4	0	0	0	0	4	13.64	0.0
P. montanense M. Chr. & Backus	6286	0	0	0	0	3	5	7	15	15	15.75	0.0
P. oxalicum Currie & Thom	6159	0	0	0	0	5	6	6	17	17	43.79	5.37
P. pinophilum Thom	6275	1	0	0	1	5	8	3	16	17	9.35	7.80
P. purpurogenum Fleroff	6026	0	0	0	0	1	1	1	3	03	53.62	6.60
P. restrictum J.C. Gilman & E.V. Abbott	6044	2	2	2	6	4	3	1	8	14	104.16	25.50
P. simplicissimum (Oudem.) Thom	6020	3	3	1	7	7	2	9	18	25	36.89	4.93
P. spinulosum Thom	6298	1	0	0	1	6	3	1	10	11	30.76	8.49
P. verruculosum Peyronel	6222	0	0	0	0	3	3	3	9	09	13.88	7.90
P. vulpinum (Cooke & Massee) Seifert & Samson	6287	0	0	0	0	5	5	5	15	15	5.85	4.25
P. waksmanii K.M. Zalessky	6158	6	3	1	10	4	5	8	17	27	19.99	5.07
Rhizopus microsporus Var. microsporus Tiegh	6217	2	3	3	08	3	1	3	7	15	0.00	0.00
Scopulariopsis brevicaulis (Sacc.) Bainier	6037	1	0	0	1	0	0	0	0	1	12.44	5.80
Syncephalastrum racemosum Cohn ex J. Schröt.	6126	11	15	9	35	0	0	0	0	35	5.88	3.70
Talaromyces minioluteus (Dierckx) Samson	6598	0	0	0	0	9	7	6	22	22	6.65	4.45
Total= 66		862	634	594	2090	1062	863	696	2621	4711		

C1 = collection 1; C2 = collection 2; C3 = collection 3; T = total isolates.

in the rainy season and 2082 in dry season. *Aspergillus flavus*, *Asp. sclerotiorum*, and *Asp. ochraceus* were the most the abundant species (**Table 1**).

The physico-chemical analyses of the soil samples revealed a pH range of 4.0 - 7.9 among the 18 collections. There was variation in the Al concentration (0.1 - 0.5 $cmol_c \cdot dm^{-3}$), Ca (0.60 - 9.60 $cmol_c \cdot dm^{-3}$), H+Al (0.8 - 2.10 $cmol_c \cdot dm^{-3}$), K (0.12 - 0.86 $cmol_c \cdot kg^{-1}$), Mg (0.7 - 2.90 $cmol_c \cdot kg^{-1}$), Na (0.25 - 0.50 $cmol_c \cdot kg^{-1}$) and P (3.70 - 280.19 $cmol_c \cdot kg^{-1}$) (**Table 2**).

The first three factors of the PCA performed with species having more than 70% frequency of occurrence explained 68.47% of the data variation; these factors were

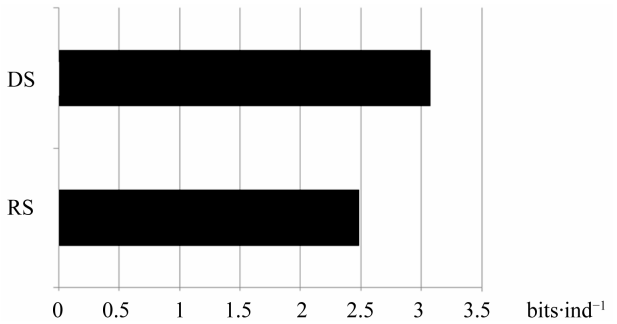

Figure 1. Diversity (bits·ind^{-1}) of filamentous fungi species in the Caatinga in the rainy season (RS) and dry season (DS). Statistical analysis based on Shannon index.

Table 2. Analysis of abiotic factors of soil samples from the area of Caatinga (Catimbau Valley, Buíque-Pernambuco, Brazil).

Collections	pH	P ($mg \cdot kg^{-1}$)	Al ($cmol_c \cdot dm^{-3}$)	Na ($cmol_c \cdot kg^{-1}$)	K ($cmol_c \cdot kg^{-1}$)	Ca ($cmol_c \cdot dm^{-3}$)	Mg ($cmol_c \cdot kg^{-1}$)	H+Al ($cmol_c \cdot dm^{-3}$)
C1	4.00	4.81	0.50	0.50	0.17	0.60	1.00	2.10
C2	6.58	280.19	0.10	0.50	0.12	5.50	0.70	1.10
C3	7.29	4.26	0.20	0.34	0.24	2.10	1.10	0.80
C4	6.26	182.59	0.50	0.30	0.38	9.10	1.90	1.60
C5	7.93	3.70	0.10	0.46	0.86	6.00	2.90	0.80
C6	4.87	35.00	0.20	0.26	0.22	1.10	1.60	0.90

C1 = collection 1; C2 = collection 2; C3 = collection 3; C4 = collection 4; C5 = collection 5; C6 = collection 6.

associated with the main abiotic soil parameters. The first factor explained 31.43% of the variation in the data, the second 21.59%, and the third 15.44% (**Figure 2**). In the first factor, temperature, Al content, and the species *Penicillium implicatum*, *P. janczewski*, *P. simplicissimum* and *P. waksmanii* were directly correlated with one another. These parameters were inversely correlated with Mg content and *Asp. ochraceus*. In the second factor, *Acremonium terricola*, *Asp. parasiticus*, *Asp. niger*, and *P. glabrum* were inversely related to *Asp. flavus*, *Rhizopus microsporus*, and Ca and H+Al levels. In the third factor, the levels of P and Na presented directly correlated to *P. lividum*, which were inversely correlated with *Asp. sclerotiorum*, pH, and K (**Figure 2**).

Dried mango and Surinam cherry leaves contained 14.28 and 7.0 g/L tannins, respectively. Higher production of tannase was obtained when mango leaves were used as the substrate for filamentous fungi in SSF. *Penicillium restrictum*, *Asp. flavofurcatus*, and *Asp. stromatoides* were the best producers, generating 104.16, 87.51, and 81.83 U/mL, respectively. Seven species did not produce tannase: *Chaetomium cupreum*, *Gliomastix murorum*, *Gongronellabutleri*, *Mortierella ramanniana*, *Neocomospora vasinfecta*, *Neosartorya fischeri*, and *R. microsporus*. When Surinam cherry leaves were used as substrate, the tannase production was quite low, with maximum activity (25.50 U/mL) also expressed by *P. restrictum*. Twelve species did not produce tannase: *Asp. sclerotiorum*, *Asp. Stromatoides*, *Asp. sulphureus*, *C. cupreum*, *Eupenicillium shaerii*, *Fusarium redolens*, *F. solani*, *Gl. murorum*, *Gon. butleri*, *M. ramanniana*, *Neoc. vasinfecta*, *Neos. fischeri*, *P. minioluteum*, *P. montanense*, and *R. microsporus* (**Table 1**).

4. Discussion

The semiarid Caatinga, compared with other Brazilian formations, has many extreme meteorological characteristics: high solar radiation, the highest average annual temperature, few clouds, the lowest relative humidities, high potential evapotranspiration, and, in particular, lower and extremely seasonal rainfall in most of the area that occurs during a very short period of the year [32]. According to Cavalcanti *et al*. [4], such environmental characteristics may favor the development of xerophilic filamentous fungi in these semiarid soils.

According Werneck [33], the Caatinga biome has received little scientific research attention relative to other tropical forests, although the Caatinga biome has high diversity that was not previously recognized. However, there remains much to be studied, especially with regard to soil fungi. This study corroborated the estimates of Werneck [33], as revealed by the high diversity of filamentous fungi in Catimbau Valley soil, especially in the dry season.

In semi-arid region of Xingó, Bahia, Brazil, Cavalcanti *et al*. [4] evaluated the diversity of filamentous fungi in soil municipalities. The authors isolated and identified 96 taxa and found that the two most representative genera were *Penicillium* and *Aspergillus*, with 31 and 18 species, respectively. The results obtained in this study corroborate those found by Cavalcanti *et al*. [4], where *Penicillium* and *Aspergillus* had the most species, with 26 and 23, respectively.

Although present in dry soils, most species of *Penicillium* prefer soils with available water [22]. According to Klich [34], who studied the biogeography of *Aspergillus* in samples of soil and leaf litter, noted that this genus occurs more frequently in desert environments, supporting our findings of large populations of *Aspergillus* Catimbau Valley soils.

Studies of the diversity of filamentous fungi in Caatinga soils are scarce. In 2006, Santiago and Souza-Motta [35] assessed the diversity of Zygomycota present in Caatinga soils in Bahia, Brazil and identified seven species, *Absidia blakesleeana* Lendner, *Abs. cylindrospora*-Hagem, *Abs. hialospora* (Saito) Lendn., *Cunninghamella elegans* Lendner, *R. microsporus* V. Thieghen, *R. oryzae* Went. & Prinsen Geerl., and *Syncephalastrum racemosum* (Cohn.) Schroet. Only five Zygomycota were found in this study, *Abs. cylindropora*, *Gon. butleri*, *M. ramanniana* var. *angulispora*, *R. microsporus* var. *microsporus* and *Syn. racemosum*.

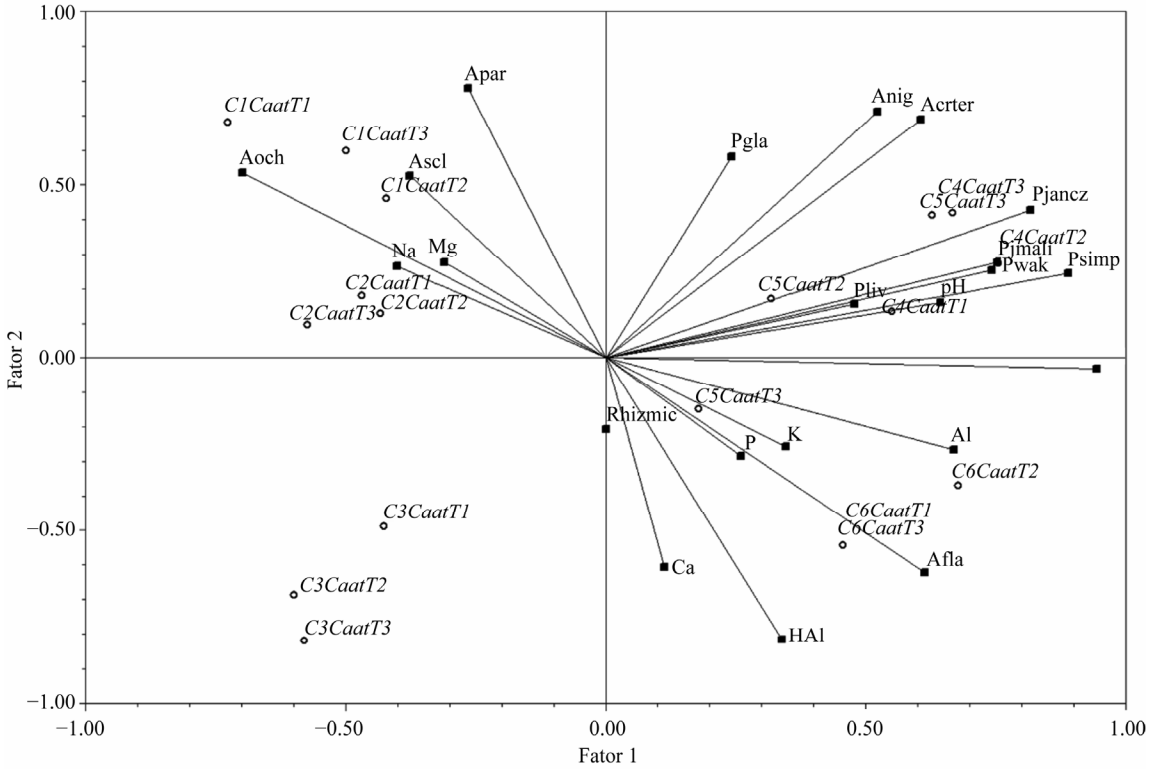

Figure 2. Two-dimensional projection of the first two factors of the principal component analysis of Caatinga area studied. (Fator 1) = Factor 1; (Fator 2) = Factor 2.

The PCA revealed the correlations, both positive and negative, between some species abundances and some physical parameters. *Aspergillus flavus*, which is common in soils [20] was the dominant species in the Caatinga biome study area. The population levels of *Asp. flavus* and *R. microsporus* var. *microsporus* were inversely correlated with those of *Acremonium terricola*, *Asp. Parasiticus*, *Asp. niger*, and *P. glabrum*, indicating that these species compete with each other, and with levels of Ca and H+Al, indicating that these abiotic parameters directly influence the competition.

The populations of *P. implicatum*, *P. janczewski*, *P. simplicissimum*, and *P. waksmanii* tended to increase with soil temperature and Al levels, while the population of *Asp. ochraceus* and Mg levels tended to decrease. The abundance of *Aspergillus sclerotiorum*, the second largest population, was directly correlated to higher pH and K level and inversely correlated to P and Na levels and the abundance of *P. lividum*. *Aspergillus ochraceus* was the third most abundant species in the soil and therefore dominant to and competing directly with *P. implicatum*, *P. janczewski*, *P. simplicissimum*, and *P. waksmanii* probably by outcompeting them for soil nutrients.

In addition to the high fungal diversity in the study area, some species had substantial potential to produce tannase at activity levels higher than those previously reported in the literature. Kumar *et al*. [12] evaluated the production of tannase by different fungal species in SSF using leaves from jujube (*Zyzyphus mauritiana*), jambul (*Syzygium cumini*), aamla (*Phyllanthus emblica*), and jowari (*Sorghum vulgare*). The authors observed a maximum yield of 69 U/g dry tissue after 96 h incubation at 30˚C for *Asp. ruber* fermenting leaves of jambul. Those results were far below those found in the present study, which was observed maximum production of 104.16 U/mL tannase by *P. restrictum* URM when mango leaves were used as substrate, indicating that mango leaves make promising substrates for SSF production of fungal tannase.

Manjit *et al*. [36] evaluated tannase production by SSF using *Asp. fumigatus* isolated from tannery effluents. As substrate, leaves from amla, jujube, jambul, *Syzygium* sp., and kikar (*Acacia nilotica*), typical Indian plants that acted as inducers of enzyme production. After optimization of production the authors observed maximum yield of tannase was 174.32 U/g at 25˚C after 96 h of incubation at pH 5.0. In this study, *P. restrictum* URM 6044 produced 104.16 U/mL under unoptimized conditions; presumably this value could be considerably improved after optimization.

In 2012, Selwal & Selwal [7] evaluated tannase production by a strain of *P. atramentosum* from tannery effluents in SSF using amla, jujube, jambul, *Syzygium* sp., and kikar leaves. The authors observed maximum tan-

nase yields of 170.75 and 165.56 U/g dry tissue from jambul and kikar leaves, respectively, incubated at 28˚C for 96 h. The form of showed of the results of this study are different from literature, as presented in U/mL, numerically lower results are to be compared with these data. The mango leaves fermented showed maximum production of 104.16 U/mL tannase by *P. restrictum* URM 6044 in a preliminary screening appear much more promising for producing tannase.

5. Conclusion

The results of this study are very promising for the production of tannase, because the filamentous fungi isolated from Catimbau Valley soil produced high levels of the enzyme using low-cost mango leaves as substrate. *Penicillium restrictum* URM 6044, *Asp. flavofurcatus* URM 6142, and *Asp. stromatoides* URM 6609 are recommended for use in subsequent studies to optimize tannase production by SSF of mango leaves.

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